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Respiratory Antibody to *Francisella tularensis* in Man

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For several years my colleagues, J. Bellanti and M. Artenstein, and I have studied the occurrence of specific antibodies in secretions of the respiratory tract, and have attempted to evaluate their biological significance (2, 3, 3*a*). This interest stemmed from the need for a simple biological marker to identify persons most likely to resist overt respiratory infection with any of several viruses. As with respiratory tularemia, the presence or titer of *humoral* antibodies to respiratory viruses is *not* synonymous with resistance to clinical disease upon infection. When these investigations were begun, it was our purpose to identify such markers. We chose to reinvestigate the occurrence of local antibody in respiratory secretions by use of more modern virological and immunological methods. Such antibodies were indeed found in nasal secretions of normal individuals. Not every individual possesses antibody to each respiratory virus; rather, detectable antibodies occur in patterns which varied from person to person (2, 3). Although there is no doubt that local antibody exists in the respiratory tract, little is known of its influence upon the pathogenesis of respiratory infections. Recently, we studied respiratory antibody to *Francisella tularensis* in man; these observations are pertinent to the questions raised by Drs. Hornick and Eigelsbach concerning effectiveness of aerosol immunization against respiratory tularemia.

Last year, with H. Dangerfield and D. Crozier of the Medical Unit, Fort Detrick, Frederick, Md., we studied respiratory antibody in 14 volunteers before and after aerosol infection with virulent *F. tularensis* (SCHU-S4 strain); this investigation will be reported in detail elsewhere. Eight volunteers were immunized percutaneously 3 months previously with LVS (tularemia vaccine, live attenuated) vaccine containing approximately 10^8 viable LVS cells per 0.1 ml. Six served as susceptible controls. One-half of each group was challenged by aerosol containing approximately 2,500 organisms; the other, with 25,000 cells. Nasal secretions were collected from these individuals by previously described methods (2) twice daily for 3 days before and for 5 days after challenge, and at weekly intervals thereafter for 6 weeks. Daily collections of nasal washings from each volunteer were pooled, concentrated approximately 10-fold by lyophilization after dialysis against distilled water, and studied for hemagglutinating antibody to polysaccharide prepared from the SCHU-S4 strain. Antibody determinations were made by the method of Alexander (1) modified for microtiter technique. Hemagglutinating antibody was measured because it is more readily detected in higher titers than are cell agglutinins (4).

Nasal antibody was indeed detected 3 months after percutaneous immunization, prior to challenge by aerosol infection (Table 1). Titers of nasal antibody ranged from 1:2 to 1:32 per 0.05 ml of concentrated nasal washing, and, for the most part, were significantly lower than those observed simultaneously in serum. There was no clear correlation between titers of antibody in serum with those found in secretions (Table 2), although too few individuals were studied to make absolute comparisons. However meager, the

TABLE 1. *Occurrence of serum and nasal antibody in eight persons to Francisella tularensis 3 months after percutaneous immunization*

Determination	Antibody[a] titer								
	2	4	8	16	32	64	128	256	512 or >
Nasal washings	3[b]	1	2	1	1				
Serum			1					2	5

[a] Reciprocal per 0.05 ml.
[b] Results expressed as number of persons with indicated titer.

TABLE 2. *Correlation between serum and nasal antibody titers 3 months after percutaneous immunization*

Nasal antibody[a]	Serum antibody[a]				
	8	256	512	1,024	2,048
2	*	*	*		
4				*	
8			*		*
16				*	
32		*			

[a] Hemagglutinin per 0.05 ml. At serum antibody dilution of 1:16 through 1:128, no hemagglutination occurred.

TABLE 3. *Development of nasal and serum antibody after aerosol infection with Francisella tularensis*

Challenge dose	Patient no.	Determination	Titer[a] pre-exposure	Titer[a] at indicated day postexposure					
				1	3	7	14	21	42
±2,500 cells.....	1	Serum	<2	—	—	<2	256	1,024	—
		NW[b]	<2	<2	<2	2	16	32	32
	2	Serum	<2	—	—	2	32	4,096	16,384
		NW	<2	<2	<2	<2	2	128	—
±25,000 cells.....	3	Serum	<2	—	—	<2	64	128	256
		NW	<2	<2	<2	<2	8	8	16
	4	Serum	<2	—	—	<2	64	512	512
		NW	<2	<2	<2	<2	4	16	8

[a] Per 0.05 ml of serum or nasal washing.
[b] NW = nasal washing.

data suggest that detectable nasal antibody occurred in persons with serum antibody titers of 1:256 or greater. The quantitative relationships between titers of nasal and serum antibodies remain to be determined.

Susceptible volunteers, when exposed to aerosols containing either 2,500 or 25,000 living cells, similarly developed nasal antibody (Table 3). Antibody was detected as early as 7 days after exposure (patient no. 1), was regularly present at 14 days, and increased in titer to levels essentially similar to those observed in percutaneously immunized personnel 3 months after vaccination (1:8 to 1:32), except for patient no. 2, whose nasal antibody titered 1:128 on the 21st day after infection. Again, titers were significantly lower than those observed simultaneously in serum. Each of the four individuals experienced respiratory tularemia, and was treated with antibiotics in the conventional fashion (6). Thus, it is clear that, irrespective of the method for infection, human beings develop nasal antibody to *F. tularensis*.

This hemagglutinating antibody of nasal secretions was found to be associated primarily with γA immunoglobulin components. Antibody-bearing secretions from each of two individuals, either the result of immunization or infection, were absorbed with goat antisera against human γA and γM immunoglobulins (Table 4). Absorption with antihuman γA immunoglobulin removed all hemagglutinin from each secretion; in contrast, absorption with antihuman γM immunoglobulin failed to remove significant amounts of antibody. Further, nasal antibody appeared to be significantly different from that of serum in the same individuals (Table 5). When high titered postimmunization or postinfection sera and nasal washings were subjected to gel filtration (Sephadex G-200), patterns of eluted

TABLE 4. *Removal of hemagglutinin from nasal secretions by specific absorption*

Antibody induced by	Subject	Antibody titers after absorption with		
		Nothing	γM	γA
Immunization.....	1	8	4	<2
	2	8	8	<2
Infection.....	1	8	4	<2
	2	8	2	<2

hemagglutinin differed between serum and secretions. The majority of antibody activity in serum was associated primarily with the γM immunoglobulins, whereas nasal antibody was found primarily in eluates containing γA immunoglobulins, and this pattern was the same after either percutaneous or aerosol infection.

These observations show that there is no significant difference in the nature of local or humoral distribution of hemagglutinin to *F. tularensis* between persons infected percutaneously or by the respiratory route. If this antibody in any way reflects resistance to overt infection (and there certainly are reasons to question this assumption), it may be properly concluded that such differences as might be effected by varying the route of vaccine administration would be only chronological. Hornick and Eiglesbach showed that the humoral antibody response following aerosol immunization is more rapid than the response to percutaneous vaccination (5). Whether local antibody appears in the respiratory tract less rapidly after percutaneous immunization is, of course, unknown, but is readily subjected to test in percutaneously immunized volunteers.

Even if respiratory antibody appears more

TABLE 5. *Partition of nasal and serum antibodies to Francisella tularensis by Sephadex gel filtration*

Sample,[a] mode infection (native titer)	γM / γA / γG										
Serum pv (256)	0	2	16	8	8	2	1	0	0	0	0
NW pv (32)	0	0	1	1	2	0	0	0	0	0	0
Serum pi (4096)	0	4	64	64	32	16	8	4	2	0	0
NW pi (128)	0	0	0	0	4	2	0	0	0	0	0
Cumulative eluate vol (ml)	35	40	45	50	55	60	65	70	75	80	85	90

[a] NW = nasal washing. The serum samples both contained immunoglobulin components γM, γA, and γG; the NW samples contained γA and γG.

promptly after aerosol immunization, there are few circumstances which demand this extraordinarily prompt immune response. Further, it is clear that, despite the presence of respiratory antibody, the immunity induced by any method of immunization can be overwhelmed by challenge with more than 10,000 virulent cells. Finally, Hornick's experience shows that administration of LVS vaccine by aerosol is not without risk of reaction (5). Indeed, to obtain optimal protection for up to 6 months, it appears necessary to administer over 10^6 to 10^8 viable vaccine cells. Approximately 80% of those receiving these doses of vaccine had, as a reaction, overt but mild respiratory tularemia. This appears to be a greater price for an additional short interval of immunity than we would be willing to pay.

Finally, it is clear that this experimentation is seriously limited by the lack of a good reproducible marker for immunity (resistance to overt infection). It is not now possible to evaluate local antibody as a marker for immunity to *F. tularensis*, although in one other respiratory infection there appears to be a good correlation between presence of respiratory antibody and resistance to infection. Experiments with parainfluenza virus type 1 in man show that persons with nasal neutralizing antibody are more resistant to challenge infection than are those without, irrespective of their humoral antibody status (Smith et al., New Engl. J. Med. *in press*). Thus, recent experimental evidence strongly suggests that detailed analysis of respiratory secretions may well provide better markers for immunity to respiratory infections. This experimental approach is not technically difficult today, and should be extended further into the problem at hand.

LITERATURE CITED

1. ALEXANDER, M. M., G. G. WRIGHT, AND A. C. BALDWIN. 1950. Observations on the agglutination of polysaccharide-treated erythrocytes by tularemia antisera. J. Exptl. Med. **91**:561–566.
2. ARTENSTEIN, M. S., J. A. BELLANTI, AND E. L. BUESCHER. 1964. Identification of the antiviral substances in nasal secretions. Proc. Soc. Exptl. Biol. Med. **117**:558–564.
3. BELLANTI, J. A., M. S. ARTENSTEIN, AND E. L. BUESCHER. 1965. Characterization of virus neutralizing antibodies in human serum and nasal secretions. J. Immunol. **94**:344–351.

3*a*. BELLANTI, J. A., M. S. ARTENSTEIN, AND E. L. BUESCHER. 1966. Ataxia telangectasia; immunologic and virologic studies of serum and respiratory secretions. Pediatrics **37**:924–933.

4. CHARKES, N. D. 1959. Hemagglutination test in tularemia; results in 56 vaccinated persons with laboratory acquired infection. J. Immunol. **83**:213–220.
5. HORNICK, R. B., AND H. T. EIGELSBACH. 1966. Aerogenic immunization of man with live tularemia vaccine. Bacteriol. Rev. **30**:532–538.
6. MCCRUMB, F. R., JR., M. J. SNYDER, AND T. E. WOODWARD. 1957. Studies on human infection with pasteurella tularensis; comparison of streptomycin and chloramphenicol in the prophylaxis of clinical disease. Trans. Assoc. Am. Physicians **70**:74–79.